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Nociceptor Response – A Review of Literature

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14. ABSTRACT

The factors contributing to heat-sensitive nociceptor activation and models for the human response to thermal pain are reviewed from the scientific literature. The response of these nociceptors to noxious thermal stimulation depends on fiber density, thermal thresholds, spatial summation, and stimulus parameters such as heating rate, intensity, and duration. These factors are examined for their influence on pain sensation and, especially, withdrawal reaction. In addition to experimental studies, published quantitative models of the nociceptive pathway are reviewed, as they will provide a guideline in developing a probabilistic model for predicting repel times due to thermal stimuli.

15. SUBJECT TERMS
Nociceptor, thermal pain, afferent nerve, heat sensitive, laser tissue heating, nerve density, neuron modeling, pain modeling

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LIST OF ACRONYMS

CNS	Central Nervous System
IENF	Intraepidermal Nerve Fiber Density
MMW	Millimeter Wave
TRP	Transient Receptor Potential
TRPV	Transient Receptor Potential Vanilloid

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EXECUTIVE SUMMARY

The factors contributing to heat sensitive nociceptor activation are reviewed from the scientific literature. This review is separated into research focusing on fiber density, thermal thresholds, spatial summation, and thermal stimulus parameters. These factors are critical to the sensation of thermal pain and to limiting the potential damage from thermal stimuli. The scope of this review includes experimental studies which have used laser stimulation to activate nociceptors since they provide the most reproducible results and more closely represent our own experimental data involving millimeter wave (MMW) exposures on humans. In addition to the experimental studies of nociceptor activation, an overview of the published quantitative models of the nociceptive pathway is also presented. Current models of thermal pain will facilitate the development of our own probabilistic model for predicting repel times due to thermal stimuli.

Studies on thermal perception have demonstrated that the variability in heat pain sensitivity between different body locations is related to differences in nerve fiber density (Mouraux et al., 2012). Skin biopsies have shown a linear relationship between nerve fiber density and distance from the spine with nerve fiber linear density decreasing from 28.6 fibers/mm in the trunk to 13.8 fibers/mm in the distal leg (Lauria et al., 1999; McArthur et al., 1998). However, there is no current histological technique to identify the proportions of Aδ to C fiber units at the same time (Schaible & Schmidt, 1996; Mouraux et al., 2012). Another method for quantifying nerve density, using the population of nerve fibers in human sural nerve trunks, has estimated the population of nerve fibers is approximately 1.4 Aδ fibers/mm^2 and 5 C fibers/mm^2 (Willis, 1985). Cutaneous nociceptors responding to thermal stimulation are largely polymodal, activated by thermal, mechanical, and/or chemical stimuli. For Aδ and C fibers approximately 12% and 50% are heat responsive, respectively (Schmidt et al., 1995; Adriaensen et al., 1983). Thermal detection thresholds of Aδ and C fibers have been estimated at 46.9±1.7 °C and 39.8±1.7 °C, respectively (Churyukanov et al., 2012). However, thresholds for thermal stimuli at the receptor level are dependent on heat sensitive transient receptor potential (TRP) channels. Transient receptor potential vanilloid, type 4 (TRPV4) receptors respond to temperatures above 27 °C; TRPV3, TRPV1, and TRPV2 respond to temperature increases above 31 °C, 43 °C, and 52 °C, respectively (Kandel et al., 2013).

Research regarding nociceptor contributions to pain sensation has employed a variety of methods to study the effects of thermal intensity, duration, and temperature rate. Measuring C fiber activity as a result of thermal stimulation found that the number of discharge spikes increased with increasing stimulus intensity (Bromm et al., 1984). Studies measuring pain thresholds and reaction times have shown that increasing stimulus intensity increases the perceived pain intensity and decreases reaction time as more Aδ fibers are recruited (Sikandar et al., 2013; Arendt-Nielsen & Bjerring, 1988a; Pertovaara et al., 1988). Increased heating rate results in increased pain perception which has been attributed to a more synchronized nerve fiber signal and greater temporal and spatial summation at the central nervous system (Iannetti et al., 2004). Studies investigating the effect of stimulus size on pain perception have demonstrated significant positive spatial summation indicating that the energy per surface area needed to produce pain decreases with increasing surface area (Pertovaara et al., 1988, Chen et al., 2010). However, studies on spatial summation with stimulus areas larger than tens of centimeters do not appear to exist.

Our aim is to develop a probabilistic model to estimate the repel times associated with specific thermal stimuli applied to various locations on the body. Existing quantitative models could be incorporated into an acceptable model, or provide guidelines for developing a new model. One such model as developed by Xu et al. (2008 and 2011) begins with the current developed at ion channels through thermally gated channels and chemically gated channels reacting to accrued thermal damage in the skin. Once this current passes a certain threshold, an action potential is generated which propagates through the nerve. To model the frequency of action potential generation, Xu et al. use a modified Hodgkin-Huxley model (Hodgkin &Huxley, 1952) of myelinated axons. This model also incorporates gate control theory providing differential processing of nociceptor inputs and uses Arrhenius integral models to determine thermal damage effects and the resulting pain sensation feedback. However, the Xu et al. model has limitations that involve the simplicity of the neuron modeling, responsiveness of the thermally active ion channels, and reliance on the squid axon response. Several computational tools for simulating networks of spiking neurons have been developed, such as the program NEURON (Brette et al., 2007). These tools may focus too heavily upon the interconnections of each neuron to be easily generalized to a specific model for thermal pain. However, by incorporating the additional flexibility of the various neuron models available in these software packages, a more accurate nociceptive model that avoids some of the shortcomings of the Xu et al. model may be developed.

1.0 INTRODUCTION

The response of heat sensitive nociceptors to noxious thermal stimulation depends on fiber density, thermal thresholds, spatial summation, and stimulus parameters such as heating rate, intensity, and duration. These factors, collected from existing literature, are reviewed as they influence pain sensation and withdrawal reaction. Studies that have attempted to model the nociceptive pathway are also examined. Experimental studies reviewed here will focus on those which have used laser stimulation to activate nociceptors unless stated otherwise. Early studies applying thermal stimulation with contact heat from thermodes or radiant heat from a light bulb proved to have many limitations (Arendt-Nielsen & Chen, 2003). One limitation was the rate of increase in cutaneous temperature, in the range of seconds, which was too slow to quantify reaction times from both Aδ and C fibers. The use of thermodes, through contact with the skin, also activates low threshold mechanosensitive fibers which modulate both nociceptive and heat information. Additionally, the amount of skin contact is not easily controlled and may alter the energy transfer when applied to areas of the body that are not flat (Plaghki & Mouraux, 2003). These disadvantages can be overcome to a large extent by the use of infrared lasers which have a long wavelength allowing near total absorption at the skin surface, independent of skin pigmentation. Other advantages include the use of a beam with highly controllable temporal and spatial profiles, no cutaneous contact, and a steep heating slope (approximately 50 °C/s) which can achieve target skin temperatures within milliseconds (Le Bars et al., 2001).

2.0 NOCICEPTORS

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. In this context, pain is an alarm system used to protect the body by triggering reactions and learned avoidance behaviors to decrease the cause of pain and limit potentially damaging consequences. Nociceptors are peripheral free nerve endings located at a depth of approximately 200 μm in the skin which serve as the first unit in a series of neurons leading to pain sensation (Figure 1) (Stoll & Greene, 1958). Nociceptors transmit mechanical, chemical, and thermal energy into action potentials through the opening of ion channels. To protect the body against thermal damage, a large number of transient receptor channels are involved in the signaling of temperature changes in the noxious range. The process of heat injury has two inversely related determinants: the higher the temperature the shorter the exposure time required to produce damage (Stoll & Greene, 1958). In order to protect against this broad spectrum of time and temperature domains, a system must be capable of detecting transient and sustained temperature changes. This is accomplished through the speed of information transfer of action potentials. These action potentials are transmitted from the peripheral sensory site to the central nervous system (CNS) through myelinated Aδ and unmyelinated C fibers. Myelinated Aδ fibers allow action potentials to travel with velocities around 10 m/s while unmyelinated C fibers have a slower conduction velocity around 1 m/s. These different conduction velocities enable the body with a double pain alarm system against noxious temperatures with Aδ and C fibers contributing to first and second pain sensations, respectively. However, experiments using rodent tail measurements have demonstrated conduction velocity decreases with increasing distance from the body indicating that conduction velocity is not constant but temperature dependent (Pincedé et al., 2012). Along with the presence of myelin, differences in conduction velocity between Aδ and C fibers have also been attributed to fiber

diameter with Aδ fibers being 2-6 µm while C fibers have a diameter of 0.4-1.2 µm (Mouraux et al., 2012). Albeit minor, this diameter dependence on conduction velocity could also indicate a further increase in conduction velocity with the confluence of peripheral fibers into larger common branches (Benoist et al., 2008).

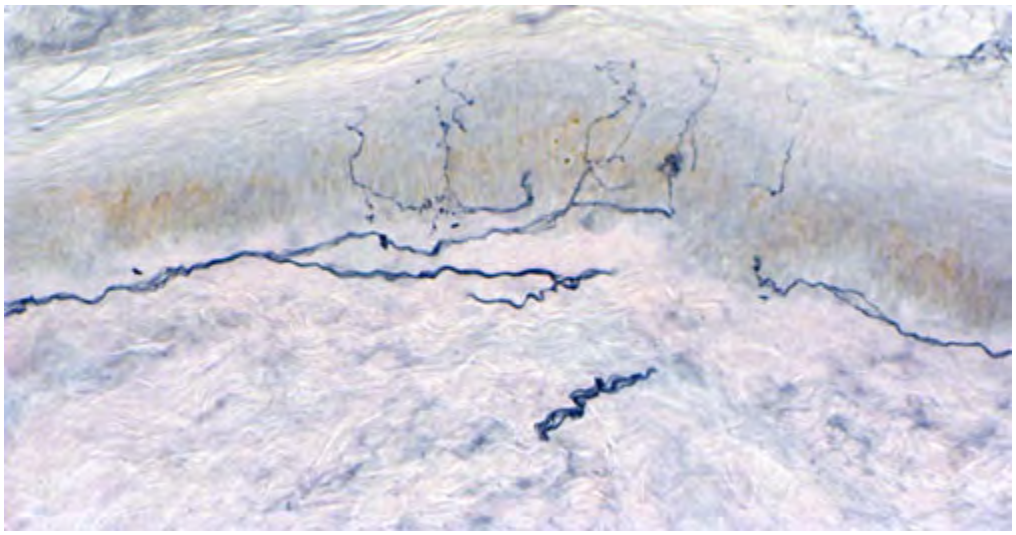

Figure 1. Intraepidermal nerve fibers demonstrated from a 3 mm punch biopsy in the leg. Note: From Advanced Laboratory Services. (n.d.) Epidermal Nerve Fiber Density (ENFD) Testing. Retrieved July 25, 2014, from http://www.advanced-lab.com/epidermal.php. Reprinted with permission.

3.0 FIBER DENSITY

The variability in heat pain sensitivity between different body locations has been attributed to differences in intraepidermal nerve fiber density (IENF) (Mouraux et al., 2012). Pinprick thresholds determined from laser stimulation have demonstrated a proximal-distal gradient indicating a possible inverse gradient in receptor density (Agostino et al., 2000). Skin biopsies have demonstrated a linear relationship between IENF density and distance from the spine with IENF linear density decreasing from 28.6 fibers/mm in the trunk to 13.8 fibers/mm in the distal leg (Lauria et al., 1999; McArthur et al., 1998). However, there is no current histological technique to identify the portions of Aδ and C fiber units (Schaible & Schmidt, 1996; Mouraux et al., 2012). Another method for quantifying nerve density, using the population of nerve fibers in human sural nerve trunks, has estimated the population of nerve fibers is approximately 1.4 Aδ fibers/mm^2 and 5 C fibers/mm^2 (Willis, 1985). Along with identifying Aδ and C fiber populations, subcategories of these fibers also exist according to their response to different stimuli. Nociceptors can be further classified as mechanoreceptors, chemoreceptors, mechanoinsensitive nociceptors, and polymodal nociceptors (Arendt-Nielsen & Chen, 2003; Xu & Lu, 2011). Cutaneous nociceptors responding to thermal stimulation are largely polymodal activated by thermal, mechanical, and/or chemical stimuli. For Aδ and C fibers approximately 12% and 50% are heat responsive, respectively (Schmidt et al., 1995; Adriaensen et al., 1983). From nerve density calculations, knowing the ratio of Aδ to C fibers, and the percentages of heat sensitive fibers, Mouraux et al. (2012) estimates the average density

of Aδ and C fibers is 7.5 and 95.2 fibers/mm^2, respectively. This estimate is much higher than previous reports with Aδ fibers at 1/mm^2 and C fibers at 2-8/mm^2 (Plaghki & Mouraux, 2003 Ochoa & Mair, 1969). While estimates of nerve fiber density are useful, the relationship between density and stimulus detection is still unclear. The fact that nerve fibers may branch after crossing the basal membrane also makes determining this relationship increasingly difficult. The contribution of branching to the probability of nerve activation and signal transmission is currently unknown.

In order to examine the hypothesis that a critical amount of afferent nociceptive information is required for perception, Mouraux et al. (2012) studied the relationship between psychophysical response, stimulus surface area, and nerve density. Using brief pulses from a CO_2 laser applied to the hand, the detection rate of Aδ fibers was calculated as a function of stimulus surface area (Figure 2). It was observed that the surface area necessary for a 50% detection rate of Aδ fibers is 1.62 mm^2. Knowing the average density of heat sensitive Aδ fibers is 7.5 fibers/mm^2, the total number of nociceptors required for 50% detection rate is calculated to be 12. Unfortunately, since the laser diameter was not small enough to alter C fiber detections, the same calculation could not be made for C fibers. Mouraux et al. also collected data on how the quality of the perception changed as a function of stimulus surface area. Results showed that for a stimulus surface area of 0.15 mm^2 most stimuli were qualified as "touch" but as the surface area increased, more stimuli were qualified as "thermal." From this result it can be inferred that a certain amount of spatial summation is necessary to evoke the thermal sensation.

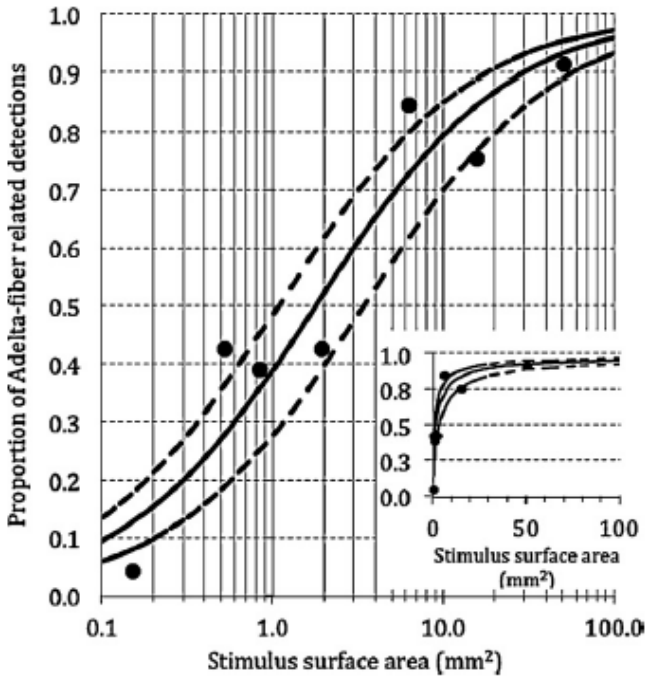

Figure 2. Proportion of Aδ fiber related detections (detections with reaction times less than 650 ms) as a function of stimulus surface area displayed on a logarithmic scale. Note: From Mouraux, A., Rage, M., Bragard, D., & Plaghki, L. (2012). Estimation of intraepidermal fiber density by the detection rate of nociceptive laser stimuli in normal and pathological conditions. *Neurophysiologie Clinique/Clinical Neurophysiology*, *42*(5), 281-291. Copyright© 2012 Elsevier Masson SAS. All rights reserved. Reprinted with permission.

4.0 THRESHOLDS

The scientific literature on thermal thresholds has primarily focused on perception and pain thresholds. Our interest here is primarily on pain thresholds as they define the lowest temperature and fiber activity needed to produce thermal pain leading to withdrawal. Early studies using lasers for thermal stimulation published fiber thresholds in terms of power output (Pertovaara et al., 1988; Arendt-Nielsen & Bjerring, 1988b). However, the variability of laser power output made these thresholds difficult to interpret and compare with other studies. With the development of infrared cameras and the use of radiometers in a closed loop feedback system to control skin temperature, thresholds can now be reported as skin temperature required for fiber activation. Since these devices measure temperature at the skin surface, and not at the nociceptor level, numerical modeling is often required to calculate the actual fiber thresholds (Marchandise et al., 2014).

In a recent publication by Plaghki et al. (2010), the pain sensation pathway is deconstructed in order to calculate true Aδ and C fiber thresholds and latencies (Figure 3). The authors discuss that during thermal stimulation the temperature increases until the fiber threshold

is reached triggering a reaction after a certain psychophysical latency has passed. This latency is composed of the time required for the nerve signal to reach CNS, interpreting and processing of the signal by the CNS, and activation of motor neurons. During this latency the temperature applied to the skin continues to increase until an apparent threshold at the time of reaction. Parameters for this experimental setup include the initial temperature, the peripheral distance the nerve signal must travel, the surface area stimulated, and the laser power. When the first three are kept constant and the laser power is varied, true thresholds and latencies can be calculated through a modified graphical determination of the heating curve. Results from this study estimated Aδ and C fiber thresholds of 44.9 and 41.9 °C, respectively. The authors estimate that the differences between apparent and actual thresholds of the fibers to be 3-8 °C depending on the rate of stimulation. This reaction time artifact results in an overestimation of the real threshold and increases with both the rate of stimulation and the length of the peripheral nerve path.

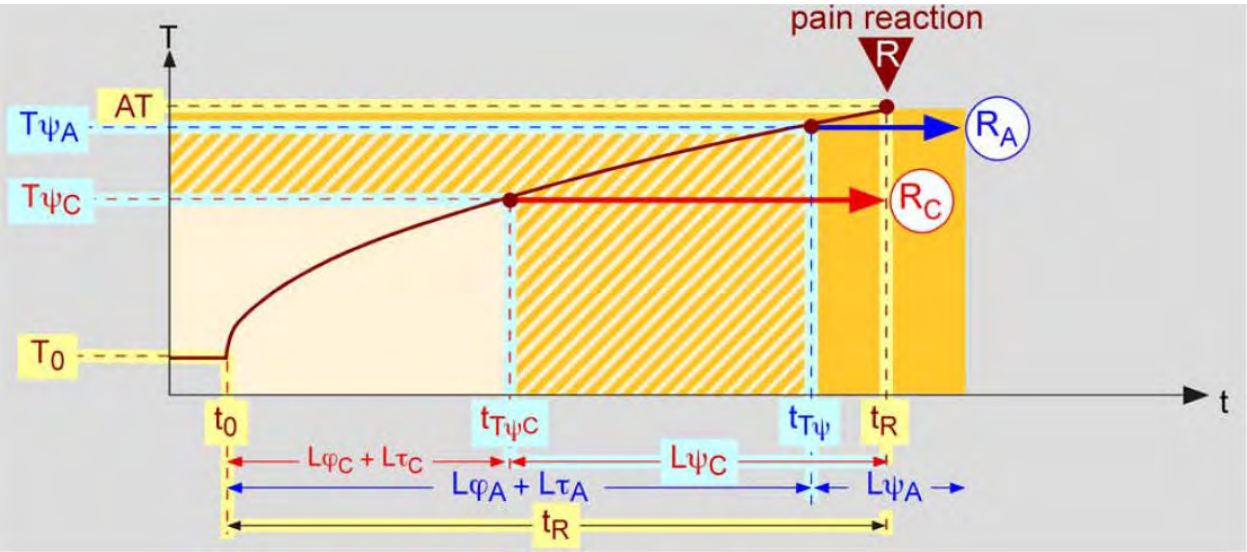

Figure 3. Theoretical analysis of nociceptive responses to heating. When the skin is exposed to a constant source of radiant heat the temperature T increases with time. Temperature increases from the initial temperature T_0 to the value AT reached at reaction. The duration of this process is the reaction time t_R. This time is organized sequentially in skin heating φ, transduction τ, and psychophysical ψ latencies. (Plaghki et al., 2010)

A study by Churyukanov et al. (2012) also estimated temperature thresholds of C and Aδ fibers using brief CO_2 laser pulses applied to the hand. The applied thermal stimuli resulted in a bimodal frequency distribution of reaction times as a result of the different conduction velocities of Aδ and C fibers. Knowing the peripheral conduction distance of the afferent nerve, a criterion of 650 ms was chosen to distinguish between C (≥ 650 ms) and Aδ (< 650 ms) fiber response (Figure 4). Using this criterion, thermal detection thresholds of Aδ and C fibers were estimated at 46.9±1.7 °C and 39.8±1.7 °C, respectively. Additionally, Churyukanov et al., investigated whether these thresholds depended on the temperature of the skin prior to the thermal stimulus. Increasing the skin temperature from a baseline value up to 38 °C just prior to thermal stimulation increased the C fiber detection threshold while the Aδ threshold remained

unchanged. This increase in threshold for C fibers was attributed to an increase in background fiber activity from lower threshold C warm receptors.

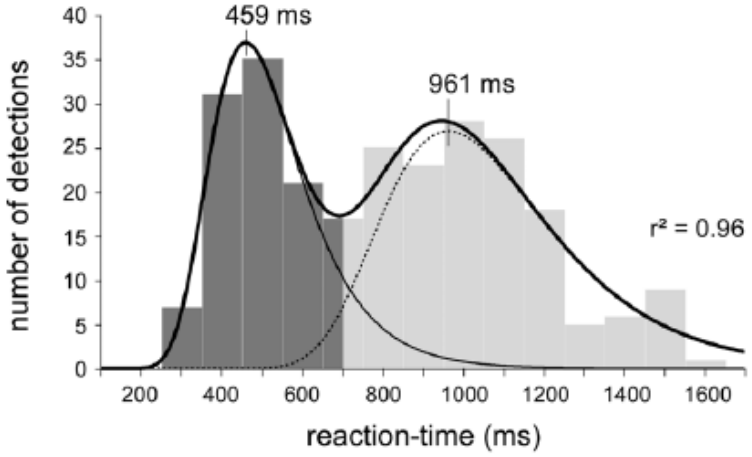

Figure 4. Frequency distribution of reaction-times to brief CO_2 laser pulses applied to the dorsum of the hand using target skin temperatures ranging from 35 to 51° (Churyukanov et al., 2012).

Fiber thresholds calculated from the forehead, hand, and foot demonstrated that thresholds were slightly higher in the forehead than the hand and foot. Thresholds have also shown to be higher for glabrous compared to hairy skin (Pertovaara et al., 1988). Differences between thresholds in the face and extremities, however, are offset by the fact that latencies are lower in the forehead due to the decreased distance to the CNS. This result is in agreement with data demonstrating a decrease in reaction time as the stimulus is moved from a distal to a proximal site on the forearm (Pertovaara et al., 1984). A decreased distance to the CNS also makes the temporal difference between first and second pain sensation less apparent at proximal locations, such as the face, where the conduction distances are shorter. To determine whether fiber thresholds are dependent on the initial skin temperature, Plaghki et al. (2010) demonstrated that increasing the skin 8 °C before stimulus resulted in a 4 °C increase in thresholds of both C and Aδ fibers. Contrary to this result, a few studies have reported a decrease in pain thresholds when the skin is heated prior to stimulation (Pertovaara et al., 1988; Arendt-Nielsen & Bjerring, 1988b, Chen et al., 2010). These authors state that warming the skin raises the skin temperature closer to the nociceptor threshold value and decreases the laser intensity needed for activation. An explanation for the difference between these conflicting studies was proposed by Plaghki et al. (2010), which involves the relative areas heated before stimulation. When a large area of skin (e.g., Plaghki et al., 2010 used areas at least 20 times greater than Petrovaara et al., 1984) is heated, information emanating from the large area to the CNS blurs the detection of a nociceptive event as compared to a smaller site. Thus, it is hypothesized that low background temperatures better facilitate the detection of a nociceptive event resulting in lower pain thresholds.

At the receptor level, temperature thresholds for thermal stimuli are dependent on heat sensitive TRP channels (Figure 5). Thermal sensation activity is derived from six types of afferent fibers, with warm receptors and heat nociceptors being relevant to thermal heating. Transient receptor potential (TRP) vanilloid (TRPV) ion channels, located in Aδ and C fibers, increase monotonically and will saturate at specific temperatures (Figure 6). TRPV4 receptors respond to temperatures above 27 °C; TRPV3, TRPV1, and TRPV2 respond to temperature increases above 31 °C, 43 °C, and 52 °C, respectively (Kandel et al., 2013). The temperature sensitivity of these TRPV channels has been found to be dependent on transmembrane voltage (Voets et al., 2004). However, it was recently shown that thermal sensitivity is determined by the channel rather than the properties of the membrane (Cao et al., 2013). Also, the channel activation by temperature follows a single exponential time course, but the temperature sensing mechanism may be coupled to agonist bindings, thus making the interaction more complicated (Yao et al., 2010). This complex interaction could explain why TRPV knockout mice still behave normally to noxious heat and because there is still a response to heat without the recognized TRPV channels, it suggests that there still may be neurons outside of Aδ and C fibers, with undefined TRP receptors, that may sense acute thermal pain (Hiura, 2009).

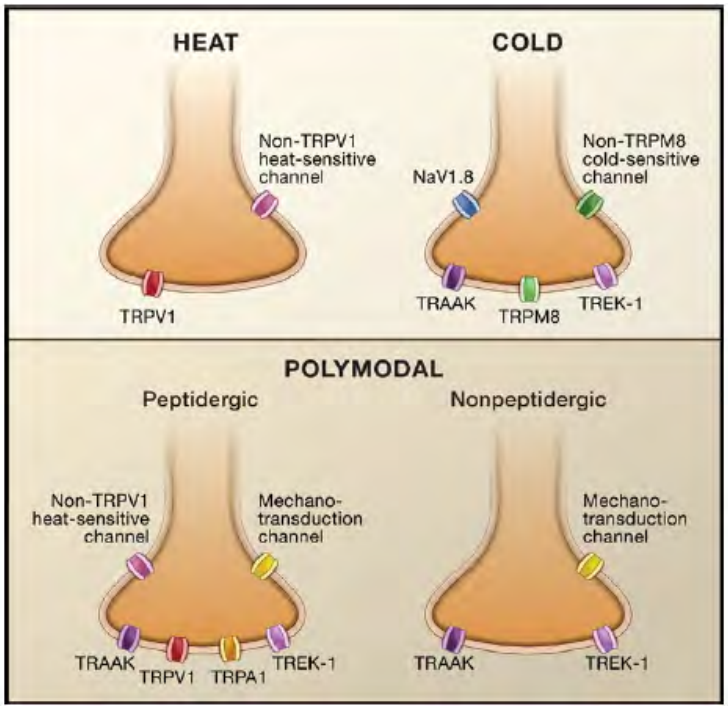

Figure 5. Schematic of ion channels located on peripheral nociceptors (Basbaum et al., 2009).

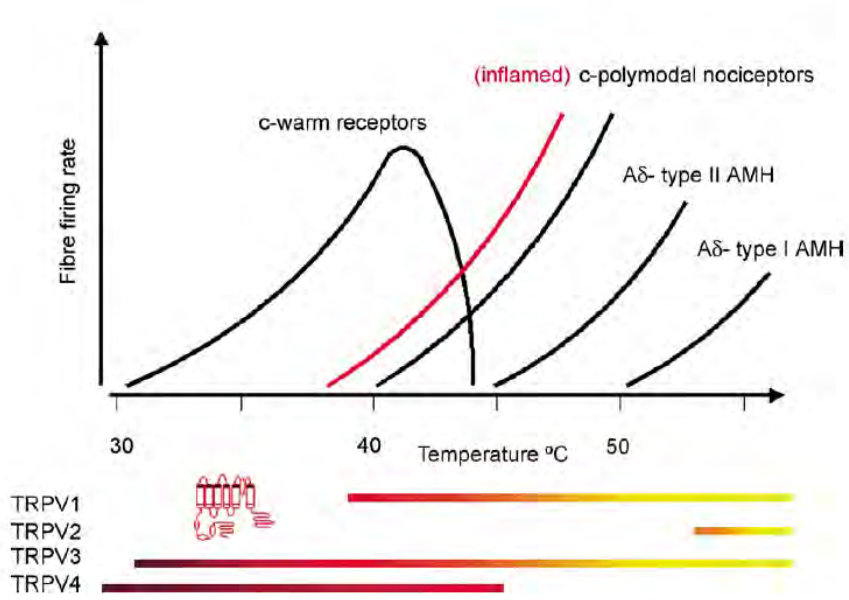

Figure 6. Fiber firing rates plotted against temperature of four types of heat sensitive ion channels in afferent nociceptors. C fibers demonstrate a leftward shift in temperature threshold in response to inflammatory mediators (red line). Activation ranges of TRPV channels are indicated by the horizontal bars below the X-axis. Note: From Benham, C. D., Gunthorpe, M. J., & Davis, J. B. (2003). TRPV channels as temperature sensors. *Cell Calcium*, *33*(5), 479-487. Copyright© 2003 Elsevier Science Ltd. All rights reserved. Reprinted with permission.

5.0 STIMULUS PARAMETERS

To understand how nociceptor activity contributes to pain sensation, a variety of methods have been employed to study the effects of thermal energy, duration, and temperature rate. An early method used microelectrodes inserted into a single peripheral nerve to record nerve fiber discharges as a result of heat stimulation (Figure 7) (Torebjork et al., 1984; Van Hees & Gybels, 1981; Yarnitsky et al., 1992). The subject's ratings of the pain sensation could also be recorded to obtain relationships between the stimulus intensity, fiber discharges, and pain ratings. Bromm et al. (1984) measured C fiber activity from the wrist following CO_2 laser stimulation and found that the number of discharge spikes increased with increasing stimulus intensity. Discharge rates as high as 75 discharges/s were recorded, but were not always accompanied by pain. However, when pain was reported nerve discharges of 60 ms or more were observed with discharge rates up to 125 discharges/s. However, a study measuring C fiber discharges from the foot demonstrated that the number of action potentials generated saturates at a frequency of 55 Hz (Olausson, 1998). Although the number of microneurology studies is limited, with even fewer using laser thermal stimuli, they still provide one of the few direct ways to record nerve fiber discharges. Additionally, data from Aδ fibers in microneurology have been numerically under represented for methodological reasons (Adriaensen et al., 1983).

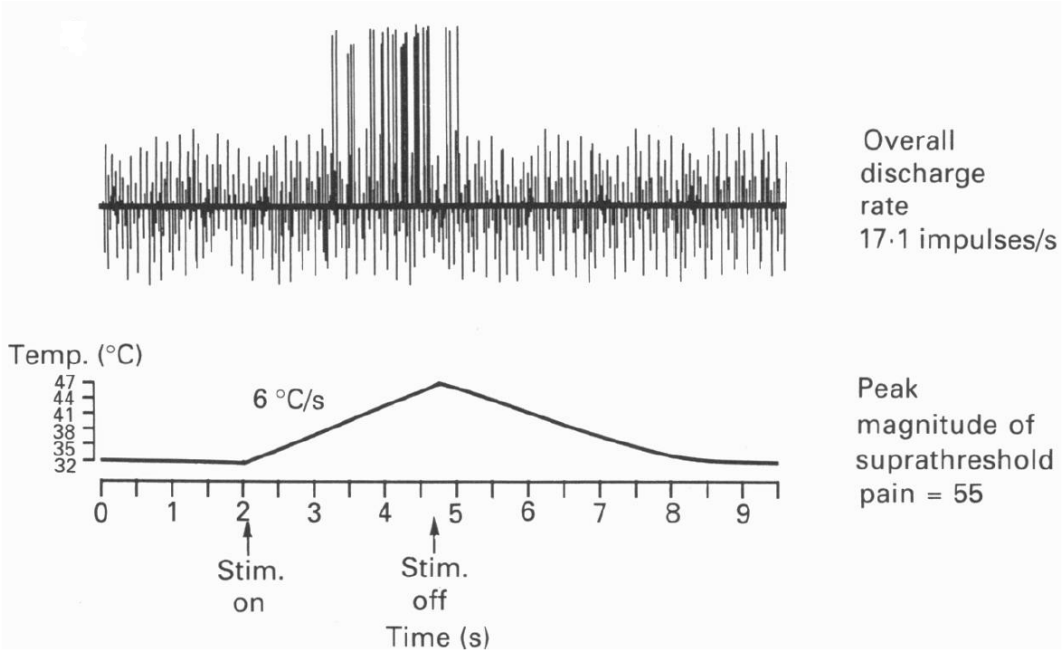

Figure 7. Discharge of a single C nociceptor in response to heat stimulation. Note: From Yarnitsky, D., Simone, D. A., Dotson, R. M., Cline, M. A., & Ochoa, J. L. (1992). Single C nociceptor responses and psychophysical parameters of evoked pain: Effect of rate of rise of heat stimuli in humans. *The Journal of Physiology*, *450*(1), 581-592. Copyright © 1992 John Wiley & Sons, Inc. All Rights Reserved. Reprinted with permission.

Studies measuring pain ratings and reaction times as a result of CO_2 laser stimulation have shown that increasing stimulus intensity increases the perceived pain intensity and decreases reaction time as Aδ fibers are recruited (Sikandar et al., 2013 Arendt-Nielsen & Bjerring, 1988a; Pertovaara et al., 1988). Increasing stimulus duration has also shown a decrease in the heat-pain threshold (Arendt-Nielsen & Bjerring, 1988b). However, increasing the stimulus duration also increases the energy delivered to the skin, therefore negating the selective assessment of duration on pain thresholds. To circumvent this, Iannetti et al. (2004) applied millisecond laser pulses of different durations without altering the total energy delivered. Applying laser stimuli of equal energy produced similar mean temperature increases, but significantly increased the temperature rates for shorter pulses. As a result, shorter pulse durations and faster heat rates caused an increase in pain ratings. Increases in pain ratings with increased heat rate were attributed to a more synchronized afferent volley leading to a greater temporal and spatial summation at the CNS. Using a translational approach to study the effects of stimulus intensity, Sikandar et al. (2013) measured afferent signals in rat second-order spinal neurons and compared them to human behavioral responses. Neurons demonstrated a graded increase in number of spikes and duration of fiber firing with increased stimulus intensity. This increase in fiber firing duration in neurons matched the time profile of skin temperature following the stimulus. Similarly, human behavioral responses to laser stimulation were also

graded with pain intensity and number of stimuli perceived in the painful range increased with increasing energy.

6.0 SPATIAL SUMMATION

Spatial summation is defined as the effect of size of stimulation, surface area on pain thresholds, and perceived intensity (Marchand & Arsenault, 2002). A positive spatial summation effect indicates either that as the area of stimulation increases the pain threshold decreases or the perceived suprathreshold intensity increases. It is also possible that both of these processes occur simultaneously as the stimulus area increases. Early work by Hardy et al. (1940) using radiant thermal stimuli concluded an absence of spatial summation with stimulus areas of 0.07-28.3 cm^2. Using contact stimuli, Stevens et al. (1974) demonstrated that warmth, but not pain, thresholds were subject to spatial summation. These authors concluded a lack of spatial summation would be an advantage since the purpose of pain is to serve as a warning no matter how small. Additionally, since tissue damage depends on the absolute temperature reached and not on the size of the heated area, they argue pain threshold should follow a similar pattern (Stoll & Greene, 1958). However more recent studies using contact heat, hot water baths, and laser stimulation have shown that both thermal thresholds and perceived pain intensity are influenced by stimulus size.

Using contact heat from 0.21-2.1 cm^2 in size, Douglass et al. (1992) investigated the effect of stimulus size on pain ratings. Additionally, to determine whether spatial summation is mediated by higher order neurons, pain ratings were compared from stimuli applied to two adjacent dermatomes and the same stimulus area in a single dermatome. Results from this study demonstrated that increasing stimulus area significantly increased pain ratings, but that pain ratings did not increase across dermatomes compared to a single dermatome. However, testing spatial summation of dermatomes is difficult since no conclusive map of human dermatomes exists and dermatome areas vary between individuals (Lee et al., 2008). Using two contact thermodes, Quevedo & Coghill (2009) investigated the concept of fill-in where overlapping neuron recruitment produces pain sensation in a non-stimulated area between stimuli. Results demonstrated that heat pain ratings increased with increasing probe distance to a maximum at 10 cm and then decreased at larger distances. The authors suggest that when two stimuli are applied at an optimal distance, overlapping neuron populations may be activated resulting in increased neuron response (Quevedo & Coghill, 2009). Studying spatial summation using contact heat from 2.25-15.36 cm^2, Defrin & Urca (1996) demonstrated a significant decrease in heat-pain threshold and increase in pain intensity with increasing stimulus size. However, when pain intensity was plotted as a function of relative threshold, it could be seen that lower heat thresholds were responsible for increases in pain intensity (Defrin & Urca, 1996). Using a hot water bath to study spatial summation, Marchand & Arsenault (2002) were able to apply a heat stimulus over a much larger area than is usually possible with other methods. Comparing the pain from immersion of the entire arm to the immersion of smaller segments of the arm demonstrated significant spatial summation. However, the effects of spatial summation could be reduced if the arm was gradually immersed in the bath. The authors suggest that this effect could be the result of recruitment of inhibitory mechanisms by the nociceptive stimuli. These

inhibitory mechanisms could also play a role in some of the early contradictory findings regarding spatial summation.

Using laser stimulation, a number of studies have also shown significant positive spatial summation. Chen et al. (2010) using stimulus areas of 1-5 mm^2 and Pertovaara et al. (1988) using stimulus areas of 10-50 mm^2 demonstrated that the energy per surface area needed to produce pain decreased with increasing surface area. To study the effects of laser stimulation on pain perception and second-order spinal neurons, Sikandar et al. (2013) conducted a translational study using both humans and rats. Results demonstrated that both species showed a clear effect of spatial summation for stimulus areas of 28-113 mm^2. In rats, more neuron firing spikes were recorded as the surface area of stimulation was increased. In humans, laser stimuli applied over larger regions resulted in a stronger pain perception. Additionally, spatial summation was shown to have an interaction with stimulus intensity indicating that the effect of stimulus size was stronger as the energy per stimulus area increased. Interestingly, reaction time also decreased with increasing stimulus size for the highest energy tested, indicating a possible reduction in threshold for Aδ fibers at larger stimulus areas. Although a positive spatial summation effect has been reported for laser stimulation studies, these stimulation areas have been on the order of tens of millimeters. While studies using contact thermodes can apply larger areas of stimulation it is also possible that spatial summation could have a limiting response as stimulation area continues to increase. However, studies on spatial summation stimulating areas larger than tens of centimeters do not appear to exist, except in the Active Denial System literature.

7.0 NOCICEPTOR MODELING

As the end goal of this nociceptor research effort is the development of a quantitative model to estimate the repel times associated with specific thermal stimuli applied to various locations on the body, this section will review published models of nociceptor activity that could be incorporated into an acceptable model, and provide guidelines for developing a new model. Experimental verification of future models will need to be made using data obtained from human nociceptors preferably with the use of laser stimulation or MMW exposures.

A quantitative model to estimate pain sensation generated by thermal stimuli of peripheral nociceptors was developed by Xu et al. (2008 and 2011) (Figure 8). This model begins with the current developed at ion channels, through thermally and chemically gated channels, in response to the temperature history of the skin. Additional ion currents taken into account are sodium, potassium, and small leakage currents by chloride and other ions. Once this current passes a certain threshold, an action potential is generated which propagates through the nerve. To model the frequency of action potential generation, Xu et al. use a Hodgkin Huxley model (Hodgkin & Huxley, 1952) of squid axons modified to represent myelinated axons. Adjustments are made for the temperature dependent membrane properties and the addition of transient potassium channels. This model also incorporates gate control theory that provides differential processing of Aβ, Aδ, and C nociceptor inputs to determine the magnitude of a stimulus to the brain. The Xu et al. model also uses Arrhenius integral models to estimate thermal damage effects on skin and resulting feedback for pain sensation. The Arrhenius equation is a simple but useful formula for the temperature dependence of reaction rates. The

integral form of the model is calibrated to allow for the temperature history of an exposed area to yield a value of 1 once a pre-determined endpoint is exceeded.

The Xu et al. model closely approximates experimental observations of mouse C fiber discharge frequency as a function of temperature. However, several limitations of this model exist as discussed by Xu et al. One of the first limitations is the use of a simple approximation between the noxious stimuli applied to nociceptors and the current generated in the ion channels without the incorporation of actual heat sensitive ion channels. Incorporation of nociceptor TRPV channels would be desirable in any future model of pain transduction. Secondly, current in thermally gated channels is modeled as a function of nociceptors with a thermal pain threshold of 43 °C. Studies on heat-sensitive TRPV channels have shown that temperature dependent ion channels react over a range of temperatures and that opening and closing of these channels is a function of temperature, voltage, and other factors (Benham et al., 2003; Voets et al., 2004). A third limitation of this model is the lack of neuron morphology and the failure to take into account the contribution of both unmyelinated and myelinated fibers. However, previous studies (Lepora et al., 2011 and Pospischil et al., 2008) have demonstrated the use of adapting the Hodgkin-Huxley model (Hodgkin & Huxley, 1952) to represent other neuron responses, which could be directly applied to improving the Xu et al. model.

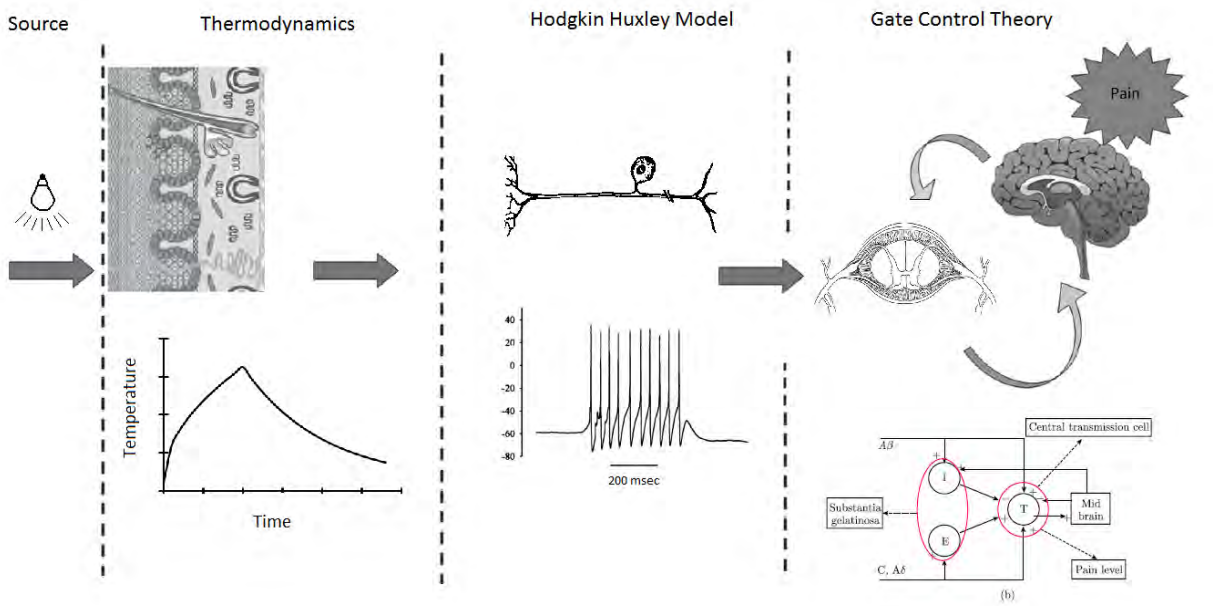

Figure 8. The skin thermal pathway. Heating of the skin is converted into electrical energy via nerve impulses. These impulses are transmitted to the central nervous system and brain where they are modulated and perceived as pain. Note: From Xu, F., Wen, T., Lu, T. J., & Seffen, K. A. (2008). Modeling of nociceptor transduction in skin thermal pain sensation. *Journal of Biomechanical Engineering*, *130*(4), 041013. Copyright © 2008 by American Society of Mechanical Engineers. Adapted with permission.

Another source of nociceptor modeling can be found in the program NEURON and other computational tools for simulating networks of spiking neurons. A review of several software implementations of neural simulations was provided by Brette et al. (2007). The common feature of these computational tools is that they provide simple to complex models for each nerve type and allow the user to define the connection topology. Feedback loops and gate control mechanisms can be incorporated through the connection diagrams. These models may focus too heavily upon the interconnections of each neuron to be easily generalized to a specific model for thermal pain, but there are published simulations of some systems that may be of use to this application. By incorporating the additional flexibility of the various neuron models available in these software packages, some of the shortcomings of the Xu et al. model may be eliminated.

In summary, we would take components from various models/studies and attempt to improve Xu's model in four areas. First, inclusion of TRPV ion channels will improve the computed membrane currents generated by thermal stimuli. Second, a more accurate

thermodynamic response of the ion channel parameters will better reflect changes occurring in the nerves at different temperatures. Third, incorporating human Aδ and C nociceptor response models will improve the fidelity over the existing mouse neuron model. Fourth, incorporating improved computational tools would allow other neuron response functions to be utilized as well as allowing the neuronal processing topology to incorporate gate control theory aspects of pain sensation. These changes would enable the model to have additional flexibility to more accurately simulate the magnitude of pain sensation generated by a thermal stimulus. This model would enable correlations with existing human repel data to improve our ability to estimate effective MMW exposure parameters.

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